DATA EVALUATION RECORD
FORAGING ACTIVITY - HONEYBEES
(NON-GUIDELINE STUDY)

1. **CHEMICAL:** Clothianidin PC Code No.: 044309

2. **TEST MATERIAL:** Clothianidin seed treatment Purity: Not reported

3. **CITATION**

Authors:	Schmuck, R., J. Keppler, P. Neumann, and C. Puth
Title:	Interim Results from a Maize Field Survey on Guttation of Maize Seedlings and Uptake of this Guttation Fluid by Honeybees
Study Completion Date:	June 21, 2009
Laboratory:	Not Reported
Sponsor:	Bayer CropScience Alfred-Nobel-Strass 50 D 40789 Monheim
Laboratory Report ID:	G202075
MRID No.:	477987-01
DP Barcode:	374484

4. **REVIEWED BY:** Joan Gaidos, Senior Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 7/14/11

APPROVED BY: John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 8/1/11

5. **APPROVED BY:** {……}, {Specialty}, OPP/EFED/ERB-{Section}

Signature: **Date:**

6. STUDY PARAMETERS

Test Species: Honeybees (species and genus not specified). Healthy colonies were used, 2 hives of *ca*. 2000 honeybees were placed next to each field (30 fields for a total of 60 hives). Each hive was in a high population growth phase, increasing the hives water demand.
Age of Test Organism at Test Initiation: Not specified. Hive colonies included 2000 honeybees (further details of the queen and the mix of sex and age were not reported).
Test Duration: Approximately 3 and 6 weeks

7. CONCLUSIONS:

In an approximately 3 or 6 week field test at two sites in Austria, two honeybee colonies were placed next to 30 fields in each region at emergence of clothianidin-treated maize seedlings and exposure to guttation fluid surveyed. The seed treatment level was not reported and the exposure levels from guttation fluid were not determined. Three key endpoints were surveyed: the frequency at which maize seedlings exuded guttation fluid; the use of exuded guttation fluid by honeybees; and the development of bee hives exposed to guttation fluid. Ten of the 30 hives in each region received water trays to differentiate effects of guttation fluids in presence or absence of alternative water sources.

Maize plants exuded guttation fluid regularly at all sites with guttation fluid observed on 96% and 76% of the assessment days at each of the two sites. The availability of an alternative water source reduced observed honeybee visitation to guttating seedlings from 16% of the assessment days at sites with no alternative water source to 4% at sites with water trays. Honeybees were observed on maize seedlings almost exclusively within 7 m of the field margin. Behaviorally impaired honeybees were observed during 5% of the assessment days with recorded symptoms ranging from apathy and lack of coordination to spasms. Daily bee mortality rates in hives provided with water trays was did not increase appreciably (not defined) during the 3-week assessment period, while mortality in hives without water trays increased in 6 out of 287 recordings (2.1%). Dead bees were analyzed and the study authors determined that residue levels suggested only a ‘casual relationship’ (data not provided). Bee mortality increased in some hives (number not reported) for 1-3 days during the 3-week study survey; however, the study authors reported that overall hive development was not adversely affected by guttation fluid. How the study authors arrived at this conclusion was not detailed.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on guttation uptake by honeybees and colony health. The study was only conducted for 3-weeks, seed treatment level was not described, test organisms were not described, exposure levels from guttation fluid were not determined, residues in dead bees were not reported, analytical methods were not detailed, statistical analysis were not described, controls were not used, environmental conditions were not

adequately described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis. Additionally, honeybee colonies were small (2000 bees) and 20% of the hives (12 of 60 hives) replaced in the first week, with 4 of the replacement hives discarded due to 'quality deficiencies', which was not further described and the impact of these replacements hives on study results were not addressed.

8. ADEQUACY OF THE STUDY

A. Classification: Core/Supplemental/Invalid

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS: This is a non-guideline test.

10. SUBMISSION PURPOSE: This study was submitted to investigate the frequency at which maize seedlings exude guttation fluid and to assess the relevance of guttation fluid to honeybees over a 3 or 6 week period. The study addressed the frequency at which maize seedlings exuded guttation fluid; the use of exuded guttation fluid by honeybees; and the development of bee hives exposed to guttation fluid. The test material, clothianidin, was apparently applied as a seed treatment; however, application details including the form and amount used were not reported.

11. MATERIALS AND METHODS

Test Material
The test material (clothianidin) was used in this study as a seed treatment; however no further details were reported.

Test Organisms
Two bee colonies with *ca.* 2000 honeybees (species not reported) per colony were placed next to each of the 30 test fields (total of 60 hives). Hives were equipped with dead bee traps to collect dead bees for residue analysis. Twelve of the 60 hives were replaced within the first week of the study due to low colony strength or they were not queen-right to support the self-maintenance of the colony (not further described). Of the replacement hives, four were discarded due to quality deficiencies and three were terminated due to shortages in forage the second week of the study (not further described).

A detailed bee health assessment was conducted every third week to determine the number of adult bees and the number of brood cells by estimating the coverage of each

comb with adult bees and brood cells. It was also noted if all brood stages and a queen were present. Less detailed assessments of colony strength and brood development were conducted on a weekly basis (not described).

An initial assessment period of 3 weeks was scheduled; however, since maize seedlings continue to exude guttation fluid beyond the 3 week period, the study was extended for a total of *ca*. 6 weeks in the north region (Baumgartenberg) while a prolonged exposure was not possible in the southern region (Jennersdorf) due to a shortage of bee attractive plants (forage). After the exposure period, the bee hives were moved to the Vienna woods with sufficient bee forage and a post-exposure assessment conducted 3 weeks after the relocation.

Seed Treatment and Crop Maintenance

In each of the two regions, 15 commercially managed maize fields were selected. Bee colonies were placed next to freshly drilled maize fields (2 colonies per field). No further details of crop maintenance, seed variety, etc, were provided.

Test Design

Experiments were conducted in 15 commercially managed maize fields in each of two representative regions for commercial maize production in Austria. One region was north of the Alps known as Baumgartenberg, and one region south of the Alps known as Jennersdorf. The study was designed to represent a worst case exposure with survey areas chosen on the basis of meteorological and pedological conditions that favored exudation of guttation fluid by maize seedlings; distant from permanent water source; landscape provided only limited nectar and pollen sources to enhance water demand of exposed bee colonies; and exposed bee colonies were in a high population growth phase, further increasing water demand of the hives. To differentiate the effects of guttation fluids in the presence or absence of alternative water sources, 10 out of the 30 hives in each region received water trays to simulate an alternative water source near the hives. Maize variety was not reported.

Three key endpoints were addressed:

- The frequency at which maize seedlings exuded guttation fluid.
- The use of exuded guttation fluid by honeybees.
- The development of bee hives exposed to guttation fluid.

Two bee colonies were placed directly adjacent to or on each of the test fields. The presence of guttation fluid and bee activity in the test fields in a 500 m^2 area per field were observed in five areas, including off-crop habitats if present. Assessment area 0 was 0-2 m around the bee hives to the edge of the field. Area 1 was 2-7 m into the field, and areas 2, 3 and 4 were adjacent to each other an additional 5, 10 and 20 m, respectively,

into the field.

The survey was initiated after emergence of the maize plants and lasted *ca.* 3 weeks. Areas were assessed by observing honeybees sitting on plants or soil surface and drinking dew or guttation water while walking slowly along the maize rows. Any observed behavioral impairments were also recorded. Three assessments of each area were conducted every other day in each field between the onset of bee activity (*ca*. 8 a.m.) until *ca*. noon. The presence and duration of guttation fluid was recorded during each field inspection and guttation water samples were collected every second inspection. Also, bee activity was assessed for 4 minute period each within six observation plots (2 m^2) between 5 and 50 m from the hives. Bee flight activity was recorded on days when guttation fluid was observed on maize seedlings and also the number of bees collecting water or at the drinking troughs in front of the hives with water trays at the start and end of the assessment.

12. REPORTED RESULTS

Signed and dated No Data Confidentiality and GLP statements were provided; a Quality Assurance statement was not provided. This study was not conducted in compliance with the Principles of Good Laboratory Practice (GLP). The data presented are based on an interim evaluation and summary of an ongoing maize field survey and a monitoring study on small beehives placed next to surveyed maize fields. The data and conclusions should be considered preliminary and the conclusions may change pending further residue analysis of sample material.

During the surveyed time period, maize plants exuded guttation fluid regularly at all sites. At the Baumgartenberg site, guttation fluid was observed on 158 days out of the 208 assessment days (no. of fields x no. of assessment days per field), equivalent to 76%. At the Jennersdorf site, guttation fluid was observed on 118 days out of the 123 assessment days, equivalent to 96%. Based on the combined data, guttation was observed on 83% of the assessment days.

Total number of assessment days and frequency of observed guttation

	Jennersdorf	Baumgartenberg	Total
Total Number of Assessment Days [No. of fields x no. of assessment days per field]	123	208	331
Frequency of Guttation [No. of assessment days at which guttation of maize seedlings was observed]	118	158	276

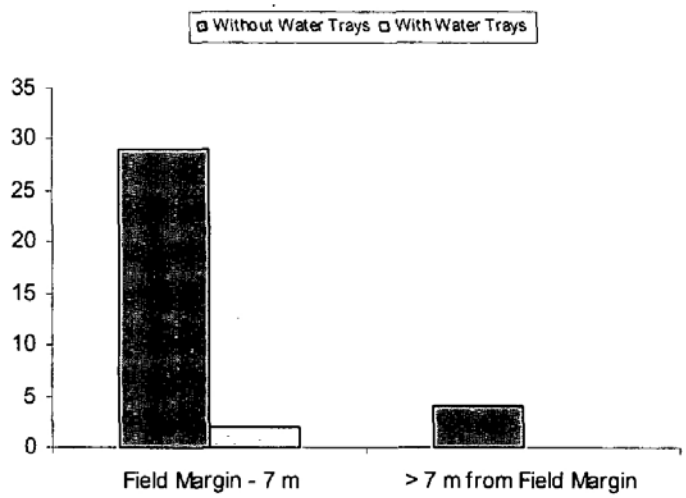
During the survey, honeybees were observed on 16% of the assessment days that guttation fluid was also observed on maize plants. The availability of an alternative water source strongly reduced visitation rate down to 4%.

Total number of assessment days with guttation and number of days when bees were observed on guttating maize seedlings

	Water Tray present?	Jennersdorf	Baumgartenberg	Total
No. of assessment days at which guttation fluid was observed on maize seedlings	+	35	65	100
No. of assessment days at which honeybees were observed on maize seedlings		2	2	4 (4%)
No. of assessment days at which guttation fluid was observed on maize seedlings	-	83	93	176
No. of assessment days at which honeybees were observed on maize seedlings		16	13	29 (16%)

When honeybees were observed on maize seedlings in the field, they were almost exclusively within 7 m from the field margin, indicating bees tend to collect water from the nearest water source. Therefore, the study authors concluded that the actual exposure is likely to be low since bee hives are not normally placed immediately adjacent to maize fields without alternative water sources.

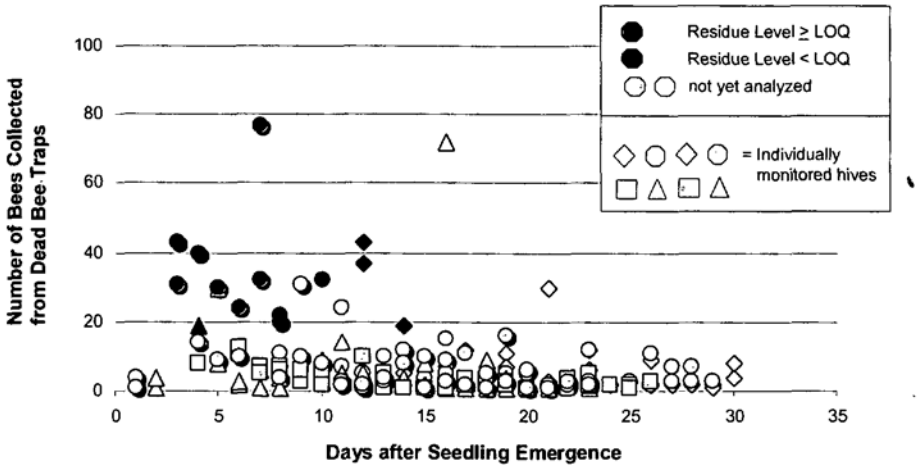
Spatial distribution pattern of honeybees observed on maize seedlings with guttation fluid



Behaviorally impaired honeybees were observed in the maize field and near the hive. Out of a total of 474 assessment days, atypical bee behavior was observed during 24 of those days, equivalent to 5%. In the majority of cases (not defined), between 1 and 4 single bees showed behavior anomalies. Recorded symptoms ranged from apathy and lack of coordination to spasms.

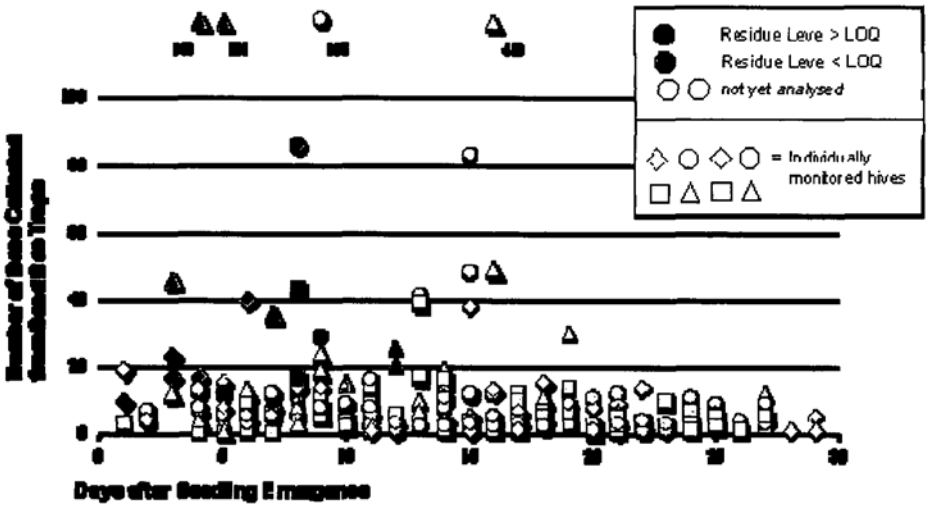
According to the study authors, the daily mortality rate of bee hives provided with water trays was not appreciably increased during the 3-week assessment period. The study author reported the number of bees collected from the dead bee traps and separated bees with clothianidin residue levels ≥LOQ and ≤LOQ (LOQ = 1 µg/kg). However, supporting tabular data, method and analysis, statistical analysis, and other pertinent details were not reported.

Daily bee mortality rate (number of dead bees collected daily from the dead bee traps) of bee hives provided with water trays (limit of quantitation = 1 µg/kg)



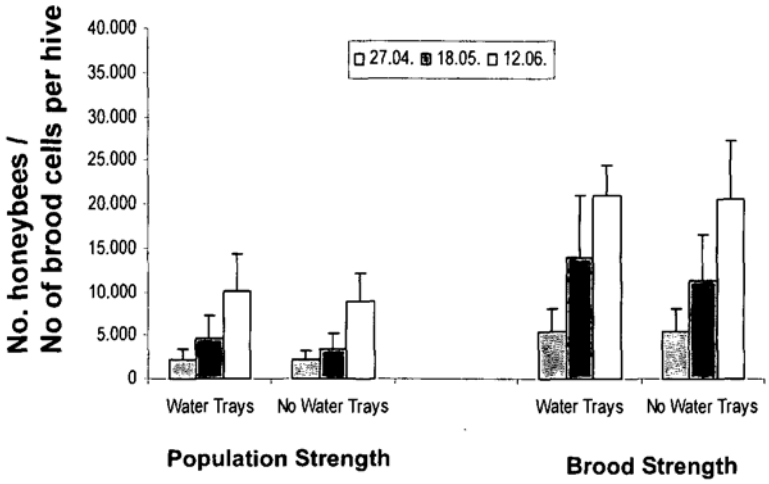
According to the study authors, during a 3 week period, daily bee mortality rates of hives wb
hich were not provided with water trays were increased by 6 out of 287 recordings (no further details or comparative results). In these cases, the authors reported that analyzed bee samples showed residue levels suggesting a 'casual relationship' (not further defined).

Daily bee mortality rate (number of dead bees collected daily from the dead bee traps) of bee hives deprived of alternative water sources (limit of quantitation = 1 µg/kg)

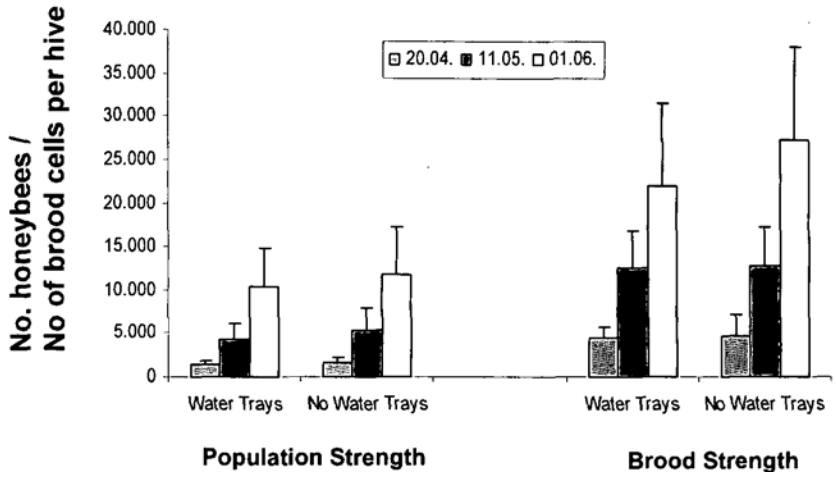


Bee mortality increased in some hives for 1-3 days during the 3-week survey. However, the overall development of the hives was not adversely affected by guttation fluid, according to the study author. All bee hives with adequate colony strength at the start of the exposure period developed well. Again, supporting tabular data, methods (i.e. definition of brood strength), statistical analysis performed, and other pertinent details were not reported.

Development of population and brood strength of bee colonies exposed to guttation fluid of maize seedlings in Jennersdorf



Development of population and brood strength of bee colonies exposed to guttation fluid of maize seedlings in Baumgartenberg



The study author reported that bee samples submitted to and analyzed by the German Bee Incident Investigation Institute in the last decade showed no clothianidin residue with the exception of one incident in 2008 which was caused by enhanced seed abrasion. Furthermore, the study authors reported a survey in Belgium which found no correlation between the intensity of exposure to clothianidin seed-treated maize fields and mortality rates of 16 apiaries which were regularly inspected between March 2004 and March 2005.

The study author concluded that guttation water of young maize plants grown from clothianidin treated seeds does not pose an unacceptable risk to honeybee colonies since:

- Under worst-case exposure conditions, effects were limited to individual honeybees when collecting guttation fluid from maize seedlings (no colony level effect).
- Only a few hives exhibited increased bee mortality, and this was only observed on occasional days during the 3-week survey.
- A reduced exposure can be expected under typical apicultural conditions in which bee hives would not be placed inside or directly adjacent to maize fields and where alternative sources of water would likely be available.

13. REVIEWER'S COMMENTS

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on guttation uptake by honeybees and colony health. The study was only conducted for 3-weeks, seed treatment level was not described, test organisms were not described, exposure levels from guttation fluid were not determined, residues in dead bees were not reported, analytical methods were not detailed, statistical analysis were not described, controls were not used, environmental conditions were not adequately described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis. Additionally, honeybee colonies were small (2000 bees) and 20% of the hives (12 of 60 hives) replaced in the first week, with 4 of the replacement hives discarded due to 'quality deficiencies', which was not further described and the impact of these replacements hives on study results were not addressed.

14. REFERENCES: None.